A CONTRIBUTION TO THE MORPHOLOGY OF PENNARIA TIARELLA McCRADY.

MARTIN SMALLWOOD.

THE following paper was undertaken at the suggestion of Dr. Charles W. Hargitt, to whose kindly criticism and suggestions I am under special obligations. I am also under obligations to Dr. C. O. Whitman for courtesies at the Marine Biological Laboratory, where in part the work was done.

The purpose of the paper is an investigation of the more fundamental morphological features of this hydroid, and the development of the Medusa and origin of the sex cells.

In the work of former students of the Pennaridæ of the United States little attention was directed to other than external characters and classification. The early contribution of McCrady (10) contains the best general account that has been given of this species. L. Agassiz's (1) description of *P. gibbosa* is equally excellent for that species; yet in neither is there any reference to features of structure, which have later assumed a degree of importance not then recognized.

Methods. — For killing and staining a variety of methods was tried, but those more generally familiar gave usually the best results. Fresh Pennaria were immersed at once in corrosive-acetic, picro-acetic, Kleinenberg, and Perenyi's fluid. The best results were obtained from corrosive-acetic and picro-acetic. An excellent differential stain was secured by staining *in toto* and then extracting with acid-alcohol. Borax carmine was found to be the best stain with material fixed in picro-acetic. There seemed to be no choice of clearing agents, either xylol or turpentine was satisfactory. Excellent whole mounts of the hydroid were made by staining for a considerable time — two days — in borax carmine, then extracting with acid-alcohol. The specimens were next cleared in clove or cedar oil for

several hours. By this process the cells could be distinguished easily in any part of the hydroid where the perisarc was not too dense.

If the parts of the colony are taken up in detail, they will be found to agree on the whole with the account of Pennaridæ given by L. Agassiz (1). The main stem arises from the hydrorhiza by a slightly geniculate course, giving off branches alternately at each bend. These branches may again divide. Each branch gradually tapers from the base, bearing on the end a single hydranth of large size. About the origin of a branch there are several annulations, from eight to twelve, and several on the branch itself near its origin. Around the base of the hydranth, which is more or less bottle-shaped,—the neck of the bottle corresponding to the distal end of the hydranth,—there is a row of tentacles, twelve in number. These tentacles round off at the end much the same as in all Tubularidæ. They are termed the "long tentacles" to distinguish them from the "short tentacles," which are more or less irregularly arranged around the oral end of the hydranth. These latter are much shorter than the long tentacles, and are arranged in two whorls. They are terminated by a knob, or globular tip, which is supplied with numerous nematocysts. There is a great deal of variation in the number of the short tentacles, due chiefly to the age of the hydranth; the older hydranth having the most. The Medusæ arise from the hydranth anywhere between the rows of tentacles. They are in direct communication with the body cavity until nearly mature, when the connection becomes closed and the Medusæ are set free.

HISTOLOGY OF PARTS.

Hydrorhiza. — There will be found in the hydrorhiza a very dense, hard perisarc on the upper surface. It is more than twice as thick here as on the opposite side and much denser. The ectoderm upon this surface of the hydrorhiza presents an almost structureless appearance. It is chiefly made up of very fine granules of protoplasm. There are scattered around in this space occupied by the ectoderm several rather large oval or

roundish bodies. In their reactions to stains they show that they are probably the nuclei of the ectodermal cells. The protoplasm is more or less vacuolated; large, round vacuoles occurring scattered through it. This is especially noticeable in older hydrorhiza. In part, the appearance of all the tissues will depend largely upon the age. A young hydrorhiza differs from an old one in the degree to which degeneration of the parts has gone. On the lower side, or side adhering to the substratum, the ectoderm is only distinguishable as a narrow layer in which there are a few scattered granules. Otherwise it appears to be structureless. It is not more than one fourth as thick here as it is on the upper side. In the young hydrorhiza, which is creeping over the eel-grass, the ectoderm contains cells which are very similar to the cells in the endoderm. The same is also true of the ectoderm in the upper side during this stage. The endoderm exhibits a cellular structure, although the cell walls are very indistinct. The cytoplasm has been broken up into small, round bodies, and the nuclei are much smaller here than elsewhere in the endoderm of the hydroid.

Hydrocaulus.—The perisarc in section in a young hydrocaulus is semi-transparent or opaque. It is thicker than when mature. In the mature hydrocaulus the perisarc is thinner, and is darker adjacent to the ectoderm than in the hydrorhiza. The ectoderm shows signs of cellular structure in the position of the nuclei and the presence of cell walls in places. As the hydrocaulus is examined nearer the hydranth the nuclei are found to belong to definite cells with definite cell walls. If the endoderm is examined in a typical section, it is very materially different from the endoderm in the hydrorhiza. The nuclei are larger and more prominent. The cytoplasm is homogeneous except for the presence of food particles.

Hydranth.—The hydranth is wholly devoid of perisarc, which terminates somewhat abruptly just below it. The cells of the ectoderm possess distinct nuclei and cell walls, but only a small amount of cell substance, judged by the reaction to stain. The cells of the endoderm are arranged in the hydranth in groups, usually there are four of them. The most conspicuous of these are the digestive cells. They are much larger than the other

cells of the endoderm, and project out into the cavity about half of their length. The nuclei are of different sizes, according to their position in the endoderm. Some of the nuclei found in the digestive cells are twice as large as those occurring in the other cells. Between these four groups of specialized digestive cells the endoderm is quite regular.

Mesoglea. — The mesoglea presented the same appearance and was of the same thickness in each region.

Origin and Development of the Medusa.

The Medusa takes its origin from the hydranth, between the rows of tentacles. There is no definite region on the hydranth from which the buds arise. However, they are more abundant near the base of the second or "longer tentacles." The number of buds which may be found upon the hydranth at one time varies. In case the hydranth is giving origin to male Medusæ there may be four or five, but usually two or three are all that will be found. The hydranths which give rise to female Medusæ generally give off only one. One exception was noted to this, but it holds true in the majority of cases. When a hydranth gives origin to more than one Medusa, they will not be in the same stage of development. I have found the two extremes on the same hydranth — the buds just forming and the mature Medusæ ready to be set free, together with several intermediate stages.

The Medusa bud arises as a simple evagination of the hydranth. The bud is hollow and supplied directly with nourishment from the circulating currents of the hydranth. As a bud becomes larger and longer, many interesting changes take place. In the mature Medusa the parts correspond to the type Hydromedusa, yet the manner in which the several layers originate is very different. Agassiz states that Pennaria develops its Medusæ in the same way as Coryne and Bougainvillia do (2). While the several parts are the same in Coryne and Pennaria, yet their origin and development are very different. The first sign of the formation of the Medusa bud is shown by a thickening of the cells of the ectoderm. This change produces a

slight papilla-like elevation on the side of the hydranth. The endoderm goes through a similar process, though not so extensive. There is no open cavity in the bud at this stage (Fig. 1). The space is entirely filled by the cells of the endoderm. The cells of the ectoderm have changed in appearance very much from those adjacent to them in the hydranth proper. These cells are the chief seat of activity, as all of the more important changes, which occur from now on, take place in them. The cells of the endoderm are secondary in importance. These results are confirmatory of the work of Dr. F. Braun (3) and contrary to that of Arthur Lang (4). The cellular structure in the ectoderm has practically disappeared at this early stage, and we have a large number of nuclei scattered in the cytoplasm. These nuclei are large and prominent. They are nearly spherical in shape. There is a very definite nucleolus, usually centrally located, which stains very deeply. The nucleoplasm has a few chromatin fibres irregularly arranged. This condition of the ectoderm is brought about in the following manner. The ectoderm, more especially at the distal end, begins to grow by a proliferation of cells, so that the thickening of the ectoderm, which takes place in the first stages in the development of the Medusa, is not so much a thickening as it is an increase in the number of cells in this region. The bud begins now to elongate until it has become partly differentiated from the hydranth (Fig. 2). The endodermal cells are smaller than at the earlier stage and enclose a cavity. The proliferation of the cells of the ectoderm continues until the bud has become about half full. It would be more accurate to say that the nuclei become more numerous than that the cells increase, because there is no indication of cells other than the presence of the nuclei and the cytoplasm in which they are imbedded. This may, however, be due to the fact that an absence of cell membrane makes difficult and indistinct the cell boundaries. While this change has been taking place, the Medusa has grown larger and longer. The

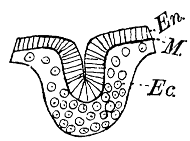

FIG. 1.

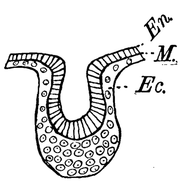

FIG. 2.

filling up of the distal end of the bud with these prominent nuclei and the surrounding cytoplasm at first forces the endoderm back, but it soon begins to force its way down through these cells in the center and around the margin of the bell. The prolongation of the cells which grow down in the center is to become the manubrium. Jointly with this formation, the endoderm is sending a layer of cells around the bell close to the ectoderm (Fig. 3). As these cells grow around, they cut off the interior mass of ectodermal cells from the outside layer, as shown in Fig. 4.

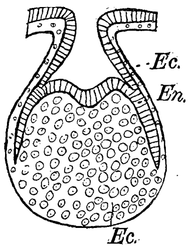

FIG. 3.

This layer of endoderm now becomes the second layer in the umbrella of the Medusa. All of the cells between the manubrium and this new layer are of ectodermal origin. The cells adjacent to this layer of endoderm show a tendency to arrange themselves in a row, the nuclei of which are smaller than those from which they have been derived. By the time the endoderm has grown around, completely separating the outer layer of ectoderm from the cells within, this row of cells has become a definite layer. This layer becomes now the lining of the bell. When the layer is completely formed, the nuclei are much smaller than they were when the layer was forming. This larger layer is more prominent in *P. cavolini;* the cells are larger and the nuclei more numerous. The mass of cells formed between the bell and the manubrium is to give rise to the

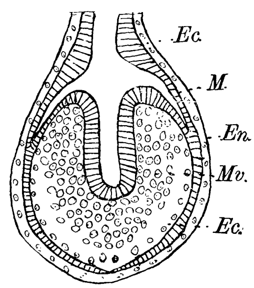

FIG. 4.

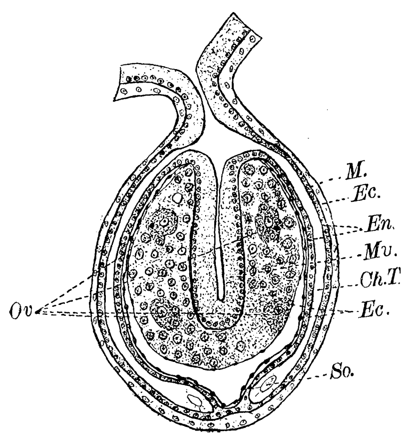

FIG. 5.

reproductive elements. Another layer is yet to be formed. As the reproductive elements continue to develop, a thin delicate membrane is produced which seems to be a differentiation from these cells (see Fig. 5). In the male it is always very delicate, while in the female it becomes more prominent. The manubrium has grown through the cells of the ectoderm while these other changes have been going on, and divides them into two equal parts when seen in section. The cells of the manubrium become differentiated until they present the same appearance as the endodermal cells in the hydranth. The cells of the endoderm which form the second layer of the bell undergo the following changes: the endoderm becomes much thicker in four regions equally distant from each other. This thickening gives rise to the chymiferous tubes by a process of cleavage and not by a fusion of two layers, as is the usual way. The splitting begins at the point where the endoderm turns in from the bell to form the manubrium (Figs. 4 and 6). This process is continued until there is a canal extending down to the lower margin of the Medusa. It is much larger at the lower end and extends farther around than do those same canals halfway up. The endoderm between the chymiferous tubes has not undergone any perceptible change during their growth. In *P. cavolini* the chymiferous tubes are much larger than in *P. tiarella*, probably because they are functional for a longer time. In this hydroid the Medusæ are not set free and these tubes are still found in the mature Medusa.

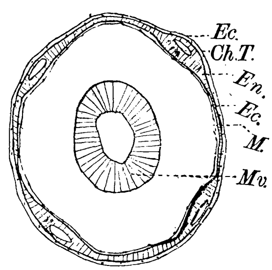

FIG. 6.

The tentacles are rudimentary and never develop. They consist simply in a thickening of the outer layer of the ectoderm. They are found at the end of the chymiferous tubes and are four in number.

The sense organs originate from the endoderm at the blind end of the chymiferous tubes. They do not show any very definite structure. In one there could be distinguished a central portion, which was more transparent than the rest, and

it did not stain so deeply. Many fine filamentous threads extended from the sense body to the walls of the tube. These threads seem to suspend it in the center. The fact that they are in such a rudimentary condition, and less highly specialized than in other Medusæ, would tend to show that their function is of a simple character, possibly used more as balancing organs than anything else. After the chymiferous tubes are formed, the further changes which take place are chiefly confined to the reproductive elements and will be explained in speaking of their development.

The description of the external morphology of the Medusa has been given so accurately by Agassiz that we can do no better than quote his exact words: "The Medusa of *P. tiarella* is one of the most remarkable of our naked-eyed Medusæ. As in the Sarsiadæ, the Medusa bud is formed among the tentacles. The chymiferous tubes never have the extraordinary thickness which is noted in Sarsia, and the cavity of the bell is hollowed out at an earlier period; the Medusa bud gradually becomes more elongated, and when mature is about one-sixteenth of an inch long." The Medusa full of eggs measures 1.2 mm. long and .7 mm. wide. Free of eggs, .9 mm. long and .5 mm. wide. He continues by saying that "the walls of the spherosome are so thin that the Medusa will often assume a quadrangular or octagonal outline with deep indentations between the chymiferous tubes. Large white eggs fill the cavity of the bell; as they increase in size they give the Medusa an opaque milky appearance. The walls of the spherosome become thinner and thinner, and when the Medusa bud has attained its full development and is ready to be separated, the walls become so thin that the Medusa is almost always distorted, either on one side or the other, by bunches of spermaries or by the eggs which have reached such a great size that four or five of them completely fill the inner cavity." The eggs are large white bodies, very opaque, .33 mm. in diameter. When they begin to segment, they look much whiter, and one can distinguish the segmenting egg by the unaided eye (2).

The opening through which the reproductive elements are to be expelled is formed after the Medusa is mature. More obser-

vations upon the Medusæ will be necessary after they become free, in order to ascertain whether a true mouth is formed. In the layers of the mature Medusa there is scarcely any cellular structure evident. The cytoplasm in the cell has disappeared, and the only structural feature present is a round body, which stains very deeply and looks as if it might be a nucleus in a very reduced condition. The chymiferous tubes have entirely disappeared except where the sense organs are found. That these tubes are functional in some of the earlier stages seems reasonably sure, because food has been found in them which was of the same character as that found in the hydranth. The connection between the hydranth and the Medusa becomes reduced with the growth of the Medusa, and finally breaks when the conditions are favorable.

The Medusæ of Pennaria seem to be in a degenerate condition, and in a sense occupy an intermediate stage between the free-swimming Medusæ and those that have lost all resemblance to this stage and are never free, as in Clava, Eudendrium, and others. The two most important reasons for thinking the Pennaria Medusæ degenerate are: first, the change which the chymiferous tubes undergo; and, second, the fact that there is no apparent mouth or circumferential canal. While the evidence is not conclusive, yet it is very suggestive and may throw some light upon other forms.

Oögenesis.

While considering the development of the Medusa, it was stated that the cells originating from the ectoderm, and which filled up the cavity between the manubrium and the bell, were destined to give rise to the reproductive elements.

If the cells are studied in such stages as are represented by Figs. 3 and 4, they will be found to be large and to possess large prominent nuclei. These cells continue to enlarge for a time, during which the cytoplasm becomes denser and more granular close to the nuclei. These may all be considered primitive ova at this stage. Five or six of these, at the most, are all that may mature into eggs. If the various cells

are carefully scrutinized at this period, it will be observed that some of them have more cytoplasm than the adjacent cells (Fig. 5). These few cells, which I have termed ova centers, become mature by the continuous increase of the cytoplasm, which is accomplished by the thrusting out of protoplasmic processes which surround the adjacent cells and absorb them into the developing egg (11). The nucleus appears to be in a state of degeneration in most of the cells adjacent to the egg. Some of the nuclei have their membrane only, while others retain all of the parts of the nucleus even after they have been taken into the egg (Fig. 7). Here some cells have just been taken into the main mass of cytoplasm. The various stages in the degeneration of the nucleus are also evident. Not all of the primitive ova are thus consumed; some will be found which have not been utilized at all. They have simply remained undeveloped and are found scattered among the mature eggs. They seem not to serve any purpose in egg formation, but represent remnants of undeveloped and unused cells.

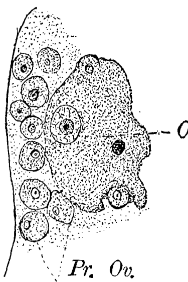

FIG. 7.

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